Bark degradation by *Aspergillus fumigatus*. Growth studies[1]

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A fungus, *Aspergillus fumigatus* Fres., which used bark as its sole carbon and energy source, was isolated. Difficulties arose in measuring fungal growth, since the hyphae and the bark could not be separated. Measurement of the weight loss of the solid material did not quantitatively estimate fungal growth. Therefore, two methods were developed to estimate fungal mass when the carbon and energy source is particulate and contributes to the parameter used as a measure of growth. They were based on determination of nitrogen either in the solid material or in the medium. The nitrogen concentration in *A. fumigatus* was found to be nearly constant throughout the growth cycle and to be independent of the carbon and nitrogen concentrations in the medium but to vary with the carbon source used.

Aspergillus fumigatus was grown at 37C as a submerged culture in salts medium with finely ground bark from *Picea abies* as sole carbon and energy source. The bark medium was heat-sterilized before inoculation with spores. The fungus utilized cellulose and hemicellulose but not lignin. Substances solubilized from the bark contributed to the growth. The yield was the same on unextracted as on water-extracted bark, although growth was delayed on the former. Growth was rapid and comparable to growth on other polymeric polysaccharides, i.e. starch. *Aspergillus fumigatus* degraded 32–40% of the polymeric part of the bark within 4 days and with an economic coefficient of about 50%.

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Nous avons isolé un champignon, *Aspergillus fumigatus* Fres., qui utilise l'écorce comme seule source de carbone et d'énergie. Nous rencontrons des difficultés dans la mesure de la croissance fongique, parce que l'hyphe et l'écorce ne peuvent pas être séparés. Des mesures de la perte en poids du matériel solide n'estiment pas quantitativement la croissance fongique. Conséquemment, deux méthodes furent développées pour estimer la masse fongique lorsque la source de carbone et d'énergie est en particules et contribue au paramètre utilisé pour la mesure de la croissance. Les méthodes sont basées sur la détermination de l'azote soit dans le matériel solide ou dans le milieu. La concentration en azote chez *A. fumigatus* est presque constante tout au cours du cycle de croissance et est indépendante des concentrations de carbone et d'azote dans le milieu, mais varie avec la source de carbone utilisée.

Nous avons fait croître en culture submergée *A. fumigatus* à 37°C dans un milieu de sels minéraux contenant de l'écorce finement moulue de *Picea abies* comme seule source de carbone et d'énergie. Le milieu composé d'écorce fut stérilisé à la chaleur avant l'inoculation avec les spores. Le champignon utilise la cellulose et l'hémicellulose mais non pas lignine. Les substances solubilisées de l'écorce contribuent à la croissance. Le rendement fut le même sur de l'écorce extraite à l'eau ou non extraite, bien que la croissance fut plus lente sur ce dernier. La croissance est rapide et comparable à celle sur d'autres polysaccharides polymériques, i.e. l'amidon. *Aspergillus fumigatus* dégrade de 32 à 40% de la partie polymérique de l'écorce en dedans de 4 jours et avec un coefficient économique d'environ 50%. [Traduit par le journal]

Introduction

The purpose of this investigation was to develop a use for bark, one of the major waste products of the paper pulp industry. Bark consists roughly of equal amounts of cellulose, hemicellulose, and lignin, but it also contains extractable substances (28, 49). There are microorganisms that are able to break down one or several of these high-molecular substances. The breakdown products formed can be used by most microorganisms as nutritional substrates. Although knowledge about bark degradation is of importance for the forest industry, little is known about how the degradation occurs in nature and about the microorganisms involved. Increased knowledge about bark-degrading microorganisms and the substances attacked under different conditions would make it possible to increase the efficiency of degradation under artificial circumstances and also to prevent or decrease unwanted degradation, i.e. when bark is used as an insulating material in road construction. The degraded bark might be used as a soil substitute or as a source for protein production. Microbial growth and activity have usually been estimated indirectly by measuring the

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evolution of carbon dioxide (2, 4, 5, 7, 14) or the weight decrease of the solid substrate (31, 38). Factors of importance for the degree of degradation are particle size (3, 36), barking method (38), nitrogen content (3, 5, 7, 13, 18), addition of lime (3), and type of bark (2, 4, 5, 38). Bark from hardwoods is more easily degraded than bark from softwoods because of the high content of resins in the latter (2, 4). However, Kuhlman (31) found no difference between extracted and unextracted bark as substrate for microorganisms. Nykvist (38), on the other hand, reported that there was a decrease in microbial activity when the bark had been stored under water.

Degradation of bark and other wood products is facilitated by the addition of nitrogen if their native content of nitrogen is poor; nitrate-nitrogen is generally more effective than ammonium-nitrogen (3). In nature, wood with a high content of cellulose is more rapidly degraded than wood with a high content of lignin (1). Mixed cultures are more efficient degraders of lignin (52, 53) and cellulose (21, 22, 27) than are pure cultures. Studies on wood components have revealed the importance of the pretreatment of the material, e.g. acid hydrolysis (26) or cutting the length of the fibers (9). Degradation of cellulose (8, 25, 29), lignin (23, 30, 48), and woody tissue (32) has recently been reviewed.

In this work, a fungus, *Aspergillus fumigatus* Fres, able to use bark as its sole carbon and energy source, was isolated. This fungus is known to degrade cellulose (43, 51) and also nitrogen-containing polysaccharides (43). Oxidation of a low molecular weight aromatic compound has also been reported (17). A commonly used method to follow fungal growth is to measure weight loss of the solid material (e.g. ref. 27). However, it was shown that this method was not relevant. Therefore, two methods based on nitrogen determinations have been developed that can be used to quantitatively measure the growth of *A. fumigatus* on bark particles in a medium. The methods have been used to measure the growth and the yields of *A. fumigatus* on unextracted bark and on bark extracted in different ways. The ability of *A. fumigatus* to degrade cellulose and lignin has also been studied.

Materials and Methods

Bark

The bark used was from Norway spruce (*Picea abies*), which had not been floated. The bark was milled; 99.9% had a particle diameter of less than 1 mm, of which 63.7% had a particle diameter less than 0.5 mm. This milled bark was a gift from Mo och Domsjö AB, Örnsköldsvik, Sweden.

The bark was used untreated (bark A), water-extracted (residue AW), or fractionated (cf. Fig. 1). Water extraction was performed by shaking untreated bark, bark A, for 24 h at 37C, in a 10% (w/v) suspension in distilled water containing 1.5% chloroform as antibacterial agent. The solid material was sedimented at 2000 × *g* (MSE Mistral 6L) for 1 h at 4C and dried for 24 h at 105C (residue AW) (Boman and Nordström, unpublished). Fractionation of bark was performed according to Norin and Winell (37). Untreated bark (580 g) was Soxhlet-extracted for 7 days with 3 liters methylene chloride, giving residue B and extract C (Fig. 1). Residue B was further extracted for 7 days with 3 liters acetone, giving residue F and extract G. A water extraction, not included in the original method, was introduced by Boman and Grennberg (unpublished). It was performed for 7 days on residue F, giving residue I and extract H. The solvents were evaporated from the extracts and the bark residues. The dried material was weighed. Methylene chloride extracted roughly 10%, acetone 14%, and water 11% (w/w) of the bark. The extracted materials varied by ±3% depending on the packing of the Soxhlet column. Hence the initial bark consisted of 65% ± 10% of residue I. The dried methylene chloride extract C was further shaken 3 times with 50 ml petroleum ether and divided into a petroleum ether insoluble (residue D) and a petroleum ether soluble part (extract E) and evaporated. Petroleum ether dissolved 78% (w/w) of the methylene chloride extract C. All fractions were stored at 4C.

Media

The minimal medium used was made from the carbon-free basal medium M63 (40) supplemented with 1% (w/v) of a carbon and energy source unless otherwise stated in the text. A nitrogen-free medium, M64, was also used; this had the same composition as M63 except that $(NH_4)_2SO_4$ was excluded. The required amount of nitrogen was added to M64 as $(NH_4)_2SO_4$. The minimal media were prepared as stock solutions containing 10 times the final concentrations of all components except for $MgSO_4$, which was autoclaved separately. The bark medium used contained residue AW unless otherwise stated. The media were solidified with 1.2% (w/v) agar. Solid rich medium (LA plates) contained LB medium (11), 2.5×10^{-3} M $CaCl_2$, vitamins (24), and 0.2% (w/v) glucose.

Materials

The cellulose used was fibrous cellulose powder CF11 (Whatman Column Chromedia). It was used either untreated or hydrolyzed (Walseth cellulose) according to Rautela and Cowling (44). The lignin was Indulin AT (Westwacco Co.) kindly donated by Dr. Ulla Westermark, Swedish Forest Products Research Laboratory, Stockholm, Sweden. According to the donor's instructions, it was dialyzed 3 times against distilled water during 24 h and freeze-dried before use. Lignosulfonic acid was kindly supplied by Mo and Domsjö AB, Örnsköldsvik, Sweden, and dehydroisoeugenol by Dr. B. Winell, Swedish Forest Products Research Laboratory, Stockholm, Sweden. The other substances were commercially available:

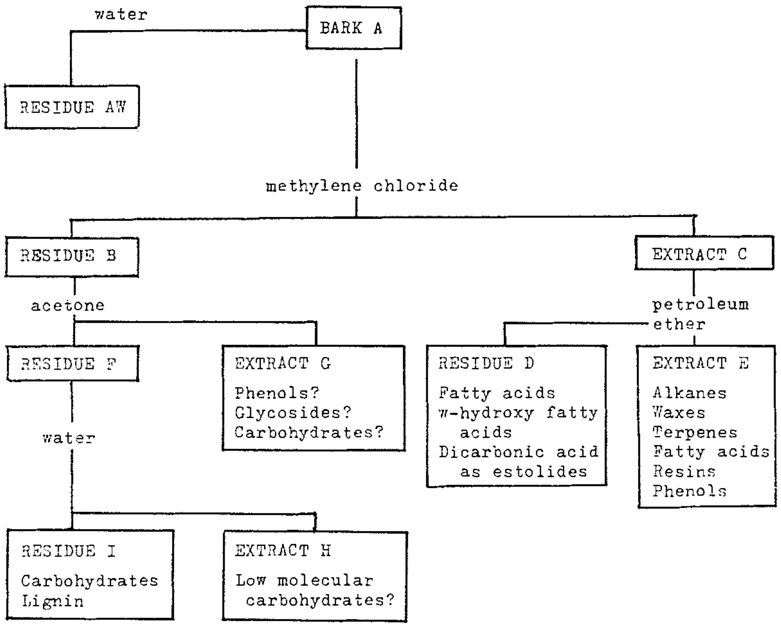

FIG. 1. Extraction of bark mainly according to Norin and Winell (37). Details of the procedure are given in Materials and Methods. Methylene chloride extracted 10% (w/w, extract C), petroleum ether 7.8% (w/w, extract E), acetone 14% (w/w, extract G), and water 11% (w/w, extract H) of bark A. Bark residues AW, B, and F were not analyzed.

phenol and vanillin (AnalaR, The British Drug Houses Ltd.), vanillic acid (Sigma Chemical Co., USA), and glucose (Mallinckrodt). As soluble starch, starch according to Zulkowsky (Merck) was used. Casamino acid came from Oxoid.

Methods for Isolation of Organisms that Utilize Bark as Sole Carbon and Energy Source

As a source of bark-degrading organisms, about 5 to 10 g of half-rotten bark was inoculated into sterile bark medium (residue AW, 1% w/v). After 1–3 days at 20 or 37C, 0.1 ml was transferred into 10 ml fresh bark medium. The process was repeated 5 times. A sample was streaked on bark agar plates and incubated. Fungi were isolated from the bark agar plates by restreaking isolated colonies 5 times on the same kind of plates. During the isolation procedure the pH of the medium was 7.0. Several bacterial and fungal species were isolated by this or similar methods (Boman and Nordström, unpublished, and Boman (15)).

Identification

One of the rapidly growing fungi isolated at 37C was further used in this study. It was identified by Centraalbureau voor Schimmelcultures, Baarn, The Netherlands, as *Aspergillus fumigatus* Fres.

Growth Conditions and Maintenance of Cultures

The fungus was kept on slants of rich medium or on bark medium and restreaked every 6 months. Spore suspensions of 50 ml were made by pouring 0.9% NaCl on a slant of the fungus grown on rich medium and collecting the suspension in a bottle. The spore suspension so prepared contained 0.5–1 × 10^7 spores/ml. Fresh spore suspensions were made every 6 months. The number of viable spores was checked at intervals by plating on LA plates. During a 6-month period the viability of the spores was constant. Spore suspensions were used as inoculum in growth experiments (56). Each inoculation was performed with a separate pipette.

Determinations of Dry Weight of the Solid Material

Triplet samples of the fungus were grown at 37C on a rotary shaker (100 rpm with an excentricity of 4 cm) in 10 ml medium in 100-ml Erlenmeyer flasks, covered with metal caps to facilitate oxygen supply. The cultures were inoculated with spores to a final concentration of 0.5–1 × 10^5 spores/ml, corresponding to a dry weight of 1–2 × 10^{-3} mg/ml. Samples were taken at intervals. Three separate cultures were analyzed for each sampling time. When an insoluble carbon and energy source was used, three flasks of control media were also analyzed. These were treated in exactly the same way as the samples except that they were not inoculated.

Immediately after the incubation period the content of each flask was filtered onto a preweighed glass filter (Whatman GF/C, diameter 4.25 mm), the flask was rinsed 5 times with 15-ml portions of distilled water, and the filter was washed another 10 times with 15-ml portions of water. The filters were dried at 105C for 24 h, allowed to cool in a desiccator at room temperature for at least 1 h, and weighed.

Measurement of the Nitrogen Content of the Solid Material

The dried sample on the glass filter was transferred to a 100-ml Kjeldahl flask and analyzed for nitrogen according to the method of Minari and Zilversmit (35), modified in the following manner. Depending on the amount of nitrogen in the sample, 4 to 20 ml of the Kjeldahl digestion reagent was added to the 100-ml Kjeldahl flask. The sample was digested with a Kjeldahl digestion apparatus model Gerhardt SRSA/6 (Grave, Sweden) and diluted with 60 to 300 ml of distilled water. Nessler reagent (3 ml) was added to 3 ml of the diluted sample. Nitrogen determinations were performed on those two samples that had the highest and the lowest dry weight for each triplet sample.

Determinations of Nitrogen Concentration in the Medium

Nitrogen concentration in the medium was determined on two growth flasks and two control flasks throughout the experiment. A 0.2-ml sample was taken from the medium at intervals and either analyzed immediately or stored at −18C before being analyzed by the following procedure. The sample was filtered by aspiration through glass wool into a pipette. The filtered sample, 0.1 or 0 05 ml, and 0.2 ml of Kjeldahl digestion reagent (35) were mixed in a Pyrex screwcap tube 11 cm long with an outer diameter of 2.1 cm and a volume of 13 ml. The tube was open during the digestion. After digestion the sample was diluted to 3 ml with distilled water and analyzed according to Minari and Zilversmit (35).

Measurement of the Phosphorus Content of the Solid Material

After weighing the filter as above, phosphorus was determined on the dried fungus or on the bark (residue AW) according to Ames and Dubin (6) in a 50-ml Pyrex test tube containing 5 ml of 10% $Mg(NO_3)_2 \cdot 6H_2O$ in ethanol. The mixture was evaporated over a strong flame. After the tube had cooled, 30 ml of 1 N HCl was added. The tube was heated in a boiling water bath for 15 min to hydrolyze to inorganic phosphate any pyrophosphate formed in the ashing procedure. The inorganic phosphate was determined by mixing 1 part of the hydrolysate with 7 parts of ascorbic-molybdate mixture (6). The absorbancy was read at 820 nm after 20 min at 45C.

Determination of Lignin and Carbohydrates

The analyses were performed by Dr. Rune Rådeström, Swedish Forest Products Research Laboratory, Stockholm, Sweden (12). The sample was hydrolyzed with 12.0 M H_2SO_4. The solid materials were filtered, washed, and dried and the amount of Klason lignin was calculated from the residual dry weight. The amount of soluble lignin was calculated from the UV extinction of the hydrolysate at 205 nm. The hydrolysate was also used for gas chromatographic analyses of the carbohydrate composition (cf. data for relative carbohydrate composition, Table 6). The absolute values were obtained by relating relative concentrations to the glucose concentration. The absolute value for glucose was obtained by using glucose oxidase (the commerical reagent Glox, Kabi Reagens, Stockholm, Sweden).

Test for Colony-formers

Substances that might influence growth were tested by adding them in various concentrations to LA plates. When the acetone-soluble extract G (cf. Fig. 1) was tested, minimal medium E of Vogel and Bonner (55) was included in the plates. The plates were spread with a spore suspension and incubated. The number of colonies obtained on the control plate without the test substance was assigned the value of 100%.

Other Methods

The rate of hyphal extension was measured either by measuring the diameter of isolated colonies after 2 days of incubation or by measuring the growth in Ryan tubes (47) after 7 days of incubation.

Absorbancies of bark medium and of lignin model substances were measured on a Hitachi Perkin-Elmer 124 Double Beam Spectrophotometer at 800–190 nm in quartz cells having a light path of 1.0 cm. Other measurements were performed on a Zeiss spectrophotometer, model PMQ11, in glass cells having a light path of 1.0 cm.

Protein was determined according to Lowry *et al.* (33).

Microscopic examinations were performed by taking two samples from the growth or control medium with a 0.1-ml pipette. The particles were counted per sample. The minimal number of particles counted per sample was 2.5×10^4 per milliliter.

Results

Isolation and Properties of a Fungus Growing on Bark

Several bacterial and fungal species were isolated by the method previously reported (15). One of the rapidly growing fungi isolated at 37C was identified as *Aspergillus fumigatus* Fres. This fungus was used for all experiments presented in this paper. *Aspergillus fumigatus* grew better at 37C than at 42C or 28C as measured by the rate of hyphal extension on bark medium. The pH of the growth medium did not change during growth. *Aspergillus fumigatus* was shown to not require growth factors. Microscopic studies of partially degraded bark revealed intimate contact between *A. fumigatus* and the bark particles.

Standard Methods for Monitoring Fungal Growth

Since the fungus and the bark particles could not be physically separated, growth could not be measured by direct dry weight determination. The often-used method of estimating growth by determining the decrease in weight of the solid material could not be used for reasons given below (cf. Fig. 9). Furthermore, changes in protein content of the bark–fungus aggregate during growth could not be determined, since substances in the bark interfered with the reagents.

Changes in the concentration of light-absorbing substances in the bark medium brought about by the fungus might have been used to measure fungal growth. However, at no wavelength between 190 and 800 nm was there any reproducible difference in absorption in media with and without growth of *A. fumigatus*. Thus

TABLE 1

Nitrogen concentrations in *A. fumigatus* and in bark

Material	Nitrogen concentration	
	No. determinations	% dry wt.
Bark A	7	0.405±0.001
Bark A, sterilized[a]	5	0.639±0.018
Residue AW, sterilized[a]	8	0.643±0.009
Residue I, sterilized[a]	6	0.652±0.004
A. fumigatus[b]	11	6.300±0.125

[a] Medium M63 containing 1% bark or residue was autoclaved and incubated for 24 h. The solid material was collected on a glass filter, washed, dried, and analyzed.

[b] *A. fumigatus* was grown to stationary phase (60 h) in M63 containing 1% (w/v) of starch, collected on a glass filter, washed, dried, and analyzed.

growth of *A. fumigatus* could not be followed by any of these methods.

Nitrogen and phosphorus determinations were possible parameters for measuring fungal growth in bark medium. Unfortunately, bark also contains nitrogen and phosphorus. The phosphorus concentration of *A. fumigatus* grown to stationary phase in glucose plus casamino acids medium was 2.0% (w/w) while that of bark (residue AW) was 0.8% (w/w). As the difference between the nitrogen concentration in the bark and in the fungus was high (Table 1), methods based on nitrogen determinations were further investigated. Determination of the nitrogen concentration in the solid material or in the medium as a parameter of growth requires that the cellular concentration of nitrogen is known and is constant throughout the growth cycle. Furthermore, since bark was the only carbon and energy source used, the fungal yield should be directly proportional to the amount of carbon and energy source utilized, i.e. the nitrogen concentration in the fungus should be independent of the concentration of the carbon and energy source in the medium, as long as this is not a growth-limiting factor. It was therefore of interest to know whether the nitrogen concentration in the fungal cell varied with different carbon sources and whether the fungal nitrogen concentration was independent of the nitrogen concentration in the medium. The growth of *A. fumigatus* was thus first studied in defined media with soluble carbon and energy sources so that the amount of cell material could be measured by dry weight determination and the nitrogen concentration could be analyzed.

Growth Studies in Defined Media

Aspergillus fumigatus was grown in glucose, in glucose plus casamino acids, and in starch medium. After 20 h of incubation, microscopic examination revealed that most of the spores had germinated and grown to short hyphae. Later these hyphae grew to form a tangle of branched mycelia and they eventually formed pellets that could easily be seen with the naked eye. After about 50 h of incubation, i.e. at stationary phase (cf. Fig. 2), spores could be seen under the microscope. They were abundant after 65 h (1×10^6 spores/ml), giving the culture a deep-green color. The dry weights increased 50- to 100-fold during the first 25 h of incubation in all media (Table 2). After this time cell mass increased linearly with time (Fig. 2). Growth was most rapid in the starch medium and slowest in the glucose medium. The yield in stationary phase was higher per milligram carbon source for cells grown in starch than for those grown in glucose plus casamino acids. Thus starch is converted to cell material more efficiently than is glucose. After the cells reached stationary phase the mass decreased. During the growth cycle the nitrogen concentration in the fungus was almost constant. The nitrogen concentration in the fungus grown in glucose plus casamino acids was higher than in starch-grown cells, 7.0 and 6.3% (w/w) respectively in stationary phase. The nitrogen concentration was lowest in the glucose-grown cells (4.5% (w/w) after 100 h) (not shown in Fig. 2).

Correlation between Cell Yield and Carbon Source Concentration

Aspergillus fumigatus was grown in media containing different concentrations of starch. The yield in stationary phase was determined. There was a linear relationship between the yield and the concentration of the carbon source

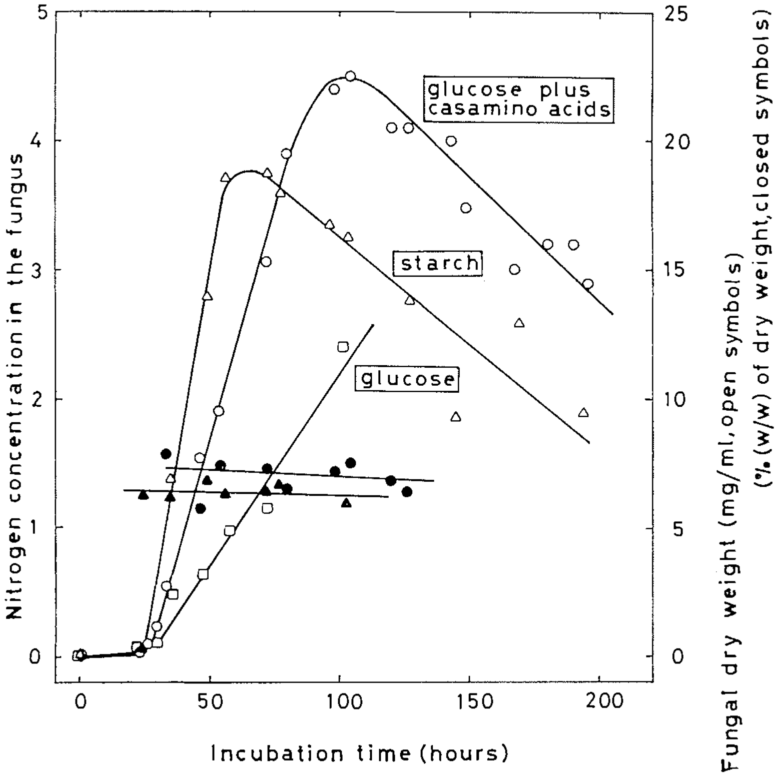

FIG. 2. Growth of, and nitrogen concentration in, *A. fumigatus* grown in defined media. Growth was measured by dry weight determinations (open symbols). The growth medium was M63, containing 1.1% (w/v) glucose, 1.1% (w/v) glucose plus 0.5% (w/v) casamino acids or 0.75% (w/v) starch. The nitrogen concentration of the solid material was determined in the same samples (closed symbols). The inoculum was 1–2 × 10^{-3} mg/ml.

TABLE 2

Early growth of *A. fumigatus*[a] in defined media

Addition to salt medium M63, % (w/v)	Dry wt. after 25 h, mg/ml	No. doublings/h
Glucose (1.1)	0.08	0.24–0.29
Glucose (1.1) plus casamino acids (0.5)	0.10	0.22–0.29
Starch (0.75)	0.09	0.22–0.29

[a] The inoculum was 1–2 × 10^{-3} mg/ml.

up to at least 6% starch (Fig. 3). In stationary phase the fungal dry weight obtained per milligram starch was 0.51 mg (i.e. the economic coefficient was 51%). A linear relationship between yield and carbon and energy source concentration was also found for cells grown in minimal medium with low glucose concentrations (0.3% to 1.1% w/v). As shown in Fig. 4 and Table 3 the nitrogen concentration of the cells was constant and independent of the yield in media in which the carbon and energy source was limited. Table 3 further illustrates that the nitrogen concentration of the fungus was independent of the nitrogen concentration in the medium.

Nitrogen Concentration in the Growth Medium during Different Growth Phases

Growth of the fungus might also be followed by measuring consumption of nitrogen in the medium freed of particles. This method was tested by growing *A. fumigatus* in starch medium. Nitrogen concentration was determined on samples from control flasks and from inoculated growth flasks. The control flasks, in which evaporation was followed, contained the same starch medium as the growth flasks. As shown in the left part of Fig. 5 there was a decrease in the nitrogen concentration in the growth medium.

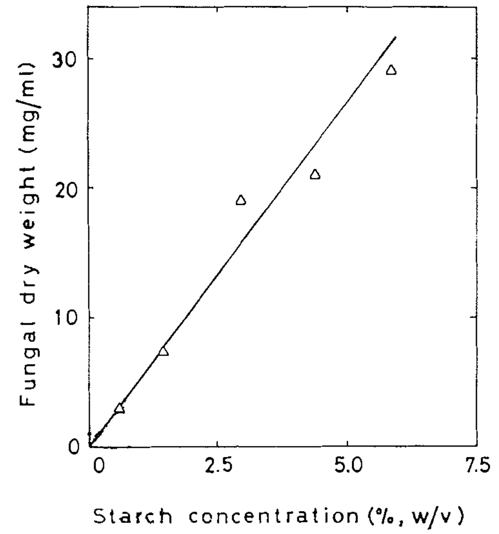

FIG. 3. Yield of *A. fumigatus* in media with different starch concentrations. The fungus was grown to stationary phase in starch medium (M64 containing 2.55 mg nitrogen/ml). Growth was measured by dry weight determinations.

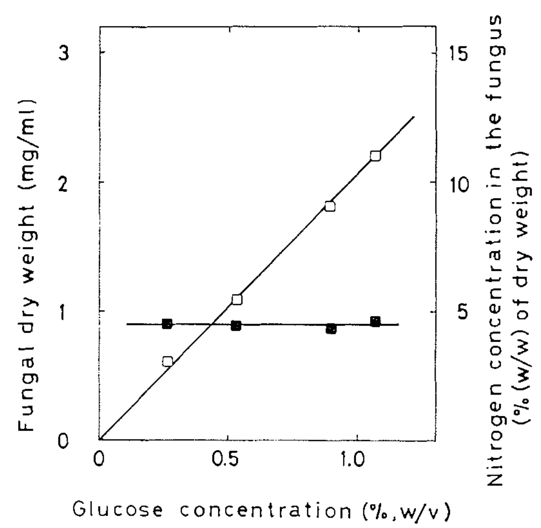

FIG. 4. Yield of, and nitrogen concentration in, *A. fumigatus* grown for 100 h in medium M63 with different glucose concentrations. Growth was followed by dry weight determinations (open symbols). The nitrogen concentration of the fungus was determined in the same samples (closed symbols).

TABLE 3

Nitrogen concentration of *A. fumigatus* grown to stationary phase in starch media with different starch and nitrogen concentrations

Addition to salt medium M64, mg/ml		Fungus	
Nitrogen[a]	Starch	Dry wt. at stationary phase, mg/ml	Nitrogen[b] concn. of the fungus, % (w/w)
0.27	7.5	3.8	6.5
0.33	10.0	5.1	6.3
0.43	7.5	3.8	6.3
0.43	10.0	5.1	6.3
2.55	5.9	3.0	6.5

[a] Added as $(NH_4)_2SO_4$.
[b] Kjeldahl analyses on the solid, dried material.

During the same time there was an increase in nitrogen concentration in the control medium. The difference in nitrogen concentration in the control and in the growth medium is illustrated in the right part of Fig. 5. In the figure the dry weights of the fungus from the same experiment are also included. As shown, there is good agreement between the growth curves obtained by dry weight determinations and by determination of the total nitrogen consumption. The fungal dry weight in stationary phase in this particular experiment was 4.5 mg/ml, corresponding to a total nitrogen consumption of 0.330 mg/ml. Thus by this method the nitrogen concentration in the fungus was found to be 7.3% (cf. below).

Derivation of Formulas for Calculating Fungal Growth

The experiments performed with soluble substrates indicated that fungal growth could be followed by determinations of nitrogen in the solid material or in the medium. When the substrate is particulate, as with bark, and cannot be separated from the fungal cells, values for dry weights and nitrogen concentrations must be corrected for contributions from the bark particles.

When the fungus grows in a bark medium, the amount of nitrogen in the solid material increases while the dry weight of the solid material decreases. The solid material consists of the fungus and the bark. The dry weight of the solid material can be expressed by the relation

$$[1] \qquad W_{FB} = W_F + W_B$$

where W_{FB}, W_F, and W_B are the dry weights (milligrams per milliliter growth medium) of the solid material (bark and fungus), of the fungus alone, and of the undegraded bark respectively.

The amount of nitrogen in the solid material is expressed by the relation

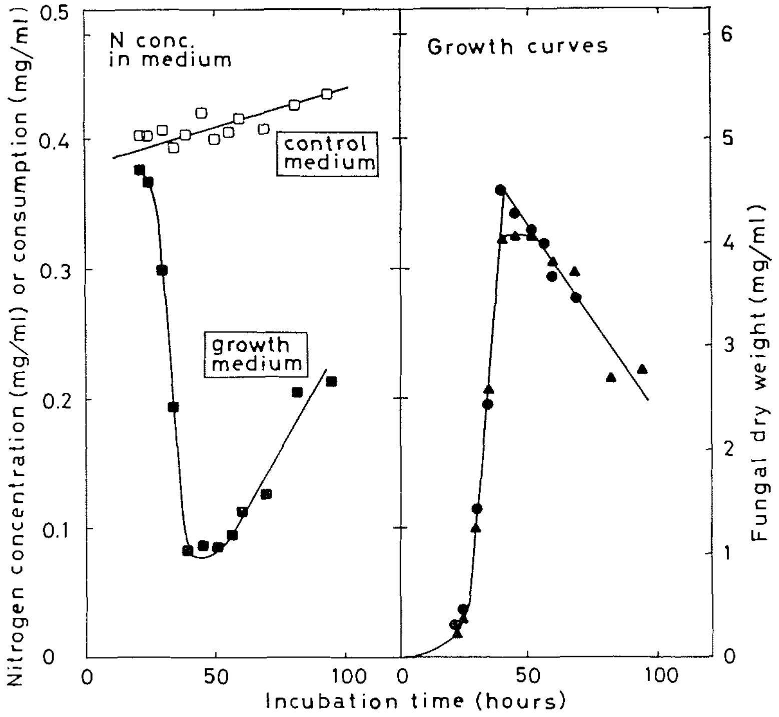

FIG. 5. Growth of *A. fumigatus* in starch minimal medium (1% (w/v) starch in M64 containing 0.340 mg nitrogen/ml). At the left, the nitrogen concentration in the growth flasks and in the control flasks during the incubation period. At the right, the difference in nitrogen concentration between the control flasks and the growth flasks (triangles), and the fungal dry weights (circles) during the incubation period.

[2] $$n_{FB} \times W_{FB} = n_{BR}W_B + n_F W_F$$

where n_{FB}, n_{BR}, and n_F are the concentrations of nitrogen (% of dry weight) in the solid material left after growth, i.e. of the mixture of fungus and bark, of the bark alone, and of the fungal cells at sampling time t respectively. To estimate fungal growth in particulate bark medium the following two assumptions were made. (1) The bark is broken down homogeneously, i.e. the nitrogen concentration in the bark left after decomposition, n_{BR}, is the same as that in undegraded bark (n_{BO}), i.e. $n_{BR} = n_B$. (2) The nitrogen concentration in the fungus (n_F) grown in bark medium is the same as the nitrogen concentration in the fungus grown on a soluble substrate. Relations 1 and 2 then give

[3] $$W_F = W_{FB}(n_{FB} - n_{BO})/(n_F - n_{BO}).$$

In starch medium the nitrogen concentration of the fungus was found to be 6.3% when the solid material was analyzed. Inserting $n_{BO} = 0.65\%$ and $n_F = 6.3\%$ (Table 1) relation 3 is written

[3*a*] $$W_F = W_{FB}(n_{FB} - 0.65)/5.65.$$

Thus if the dry weight and the amount of nitrogen in the solid material after growth are determined, the fungal weight can be calculated from relation 3.

Growth can also be calculated by measuring the consumption of the nitrogen source from the growth medium. The amount of nitrogen assimilated by the fungus, N_F, during time t is expressed by the relation

[4] $$N_F = (N_0 + N_S) - N_t.$$

N_0 and N_t are the amounts of nitrogen in the growth medium at times 0 and t, respectively, and N_S is the amount of nitrogen which has been solubilized from the bark by fungal activities. N_F and N_S can be expressed by the relations

[5] $$N_F = W_F \times n_F.$$

[6] $$N_S = W_S \times n_{BS}$$

where W_S is milligrams dry weight and n_{BS} is the nitrogen concentration of the bark solubilized by the fungus during time t. W_S is related to W_F by the relation

$$W_S = (1/k) \times W_F \tag{7}$$

where k is the economic coefficient, i.e. the amount of cell material that is obtained when one weight unit of the carbon and energy source is completely consumed by the fungus.

Relations 4, 5, 6, and 7 give the relation

$$W_F = (N_0 - N_t)/[n_F - (n_{BS}/k)]. \tag{8}$$

Three assumptions are necessary for the practical use of relation 8. These are assumptions 1 and 2 (cf. above) and an assumed value of k, chosen as 0.5 (i.e. the economic coefficient of starch is 51%). The value of n_{BS} is 0.65, i.e. equal to n_{BO}. The value of n_F is 7.3 (cf. Fig. 5), which is higher than the corresponding value in relation 3a; the Kjeldahl method underestimates the nitrogen content in e.g. purines and pyrimidines (16). Relation 8 is then transformed to relation

$$W_F = (N_0 - N_t)/6.0. \tag{8a}$$

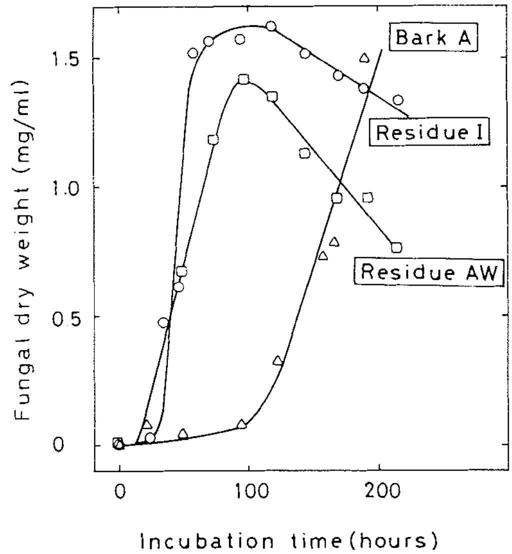

FIG. 6. Growth of *A. fumigatus* on bark treated in different ways. Bark (100 mg) was autoclaved with 10 ml of medium M63. Inoculated samples and controls were incubated. Samples and controls were harvested for dry weight determinations and nitrogen analyses of the solid material. The average dry weight values for the controls were 61.3 ± 3.5 mg (bark A), 71.7 ± 2.5 mg (residue AW), and 84.1 ± 1.5 mg (residue I) respectively. The average dry weights of the solid material in the inoculated samples decreased in comparison with the controls, and the nitrogen concentration increased (cf. Fig. 9). The fungal yields were calculated from the nitrogen and dry weight values using relation 3a.

Thus if the amount of nitrogen is measured in the medium before (N_0) and after (N_t) growth, the fungal weight can be calculated.

Growth in Bark Medium

Aspergillus fumigatus was grown in a medium containing unextracted bark (bark A), residue AW, or residue I (cf. Fig. 1). Growth was followed by nitrogen and dry weight determinations of the solid material in the samples and fungal cell mass was calculated from relation 3a, (Fig. 6). Microscopic examination after 29 h of incubation in the medium containing residue I revealed that most of the spores had germinated and had given rise to short hyphae. Fewer hyphae were seen than in starch medium. Later most hyphae were seen in intimate contact with the bark particles. The fungus kept the bark particles together. Pellets like those seen in starch medium were not observed. After about 70 h of incubation spores could be seen under the microscope and they were abundant after 96 h, i.e. when stationary phase was reached. The maximal number of spores found in the bark medium (3×10^7 spores/ml) was 100 times that found in starch medium per milligram fungus obtained. After the first 30 and 100 h of growth the cell mass increased linearly with time on extracted (residue AW or I) and unextracted bark (bark A) respectively (Fig. 6). The growth rate was the same in the three media. Residue I consists of high molecular weight polymeric substances which failed to promote growth of bacteria (tested with *Escherichia coli* and *Pseudomonas aeruginosa* (Grennberg, personal communication)). Since the preparation of all media included a sterilization step, growth-promoting components might be extracted from the high polymeric substances. It was shown that these substances solubilized from residue I supported less than 1% of normal growth. Thus the only available carbon and energy source in residue I media was the polymeric residue I. The fungal yields on residues AW and I were 0.14 ± 0.01 and 0.16 ± 0.01 mg/mg residue respectively, as determined on dried solid material. On unextracted bark, bark A, the fungal mass after 170 h of incubation was 0.15 ± 0.06 mg/mg bark. Growth on residue I was also measured in the growth medium by nitrogen determinations (Fig. 7). From the peak value of the right part of Fig. 7, relation 8a gives a yield of 0.20 mg/mg bark. The yield of *A.*

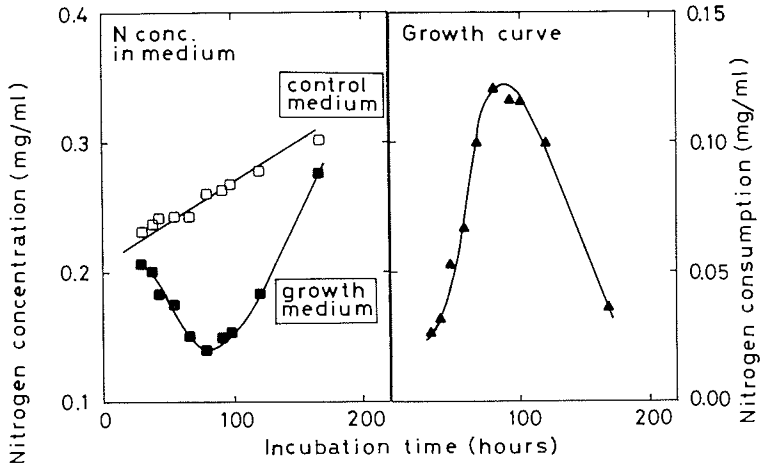

FIG. 7. Growth of *A. fumigatus* in bark medium (1% (w/v) residue I in medium M64 containing 0.205 mg nitrogen/ml). At the left, the nitrogen concentration in the growth flasks and in the control flasks during the incubation period. At the right, the difference in nitrogen concentration between the control flasks and the growth flasks during the incubation period.

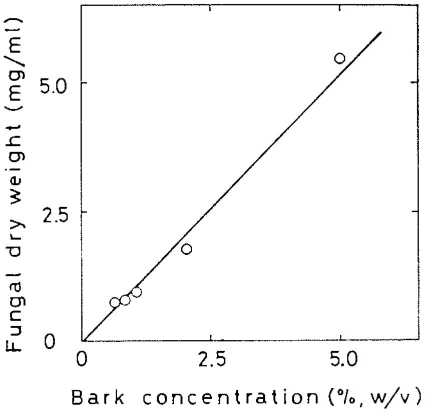

FIG. 8. Yield of *A. fumigatus* in media with different bark concentrations (residue AW). The fungus was grown for 170 h in bark medium (M64 containing 2.55 mg nitrogen/ml). Samples and controls were analyzed for dry weight and amount of nitrogen in the solid material. The fungal yields were calculated from the nitrogen and dry weight values using relation 3*a*.

fumigatus was found to be directly proportional to the amount of bark (residue AW) added to the medium (Fig. 8). The relation holds for concentrations up to at least 5% bark in the medium. Higher concentrations have not been quantitatively investigated, but growth has been observed even in media containing 12.5% of residue I.

During the growth of *A. fumigatus* on bark particles the weight of the solid material decreases. The weight decrease of the solid material and the increase in the nitrogen concentration of the solid material was followed (Fig. 9). The weight decrease was not linearly correlated to the increase in nitrogen concentration in the solid material. In the very early growth phase there was even a slight increase in weight of the

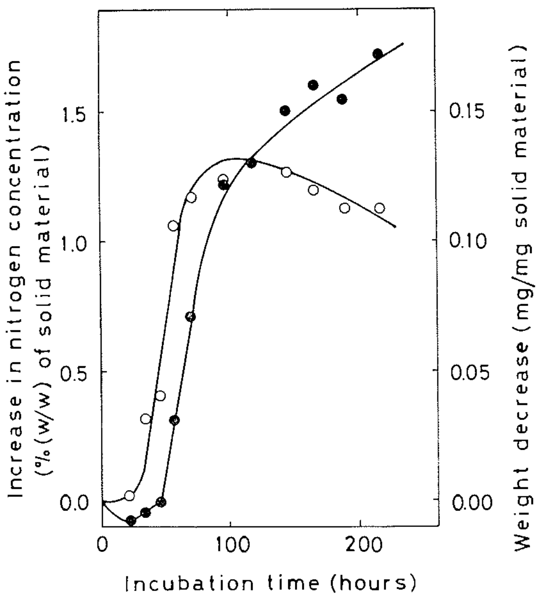

FIG. 9. Growth of *A. fumigatus* on residue I. The weight decrease of the solid material compared with the increase in nitrogen concentration in the solid material. The experiment was the same as that shown as circles in Fig. 6.

TABLE 4

Yield of *A. fumigatus* after stationary phase was attained in medium M63 containing 1% of different bark fractions or other substrates

Carbon and energy source	Fungal yield W_F,[a] mg/mg carbon and energy source	Time to achieve stationary phase, h	Nitrogen concn. of carbon and energy source, n_{BO},[b] % (w/w)
Bark A	≥0.15	>200	0.64
Residue AW	0.14	100	0.64
Residue I	0.16	100	0.65
Residue D	<0.01	>170	0.13
Extract E	0.08	100	<0.01
Extract G	No growth[c]	Not tested	Not tested
Extract H	0.28 (0.43[d])	60–70	Not tested
Glucose plus casamino acids	0.45[d]	90	<0.01
Starch	0.51[d]	80	<0.01
Glucose	≥0.21[d]	>100	<0.01
Cellulose[e]	0.02	>260	0.01
Lignin[f]	No growth[g]	>220	<0.01

[a] Growth was measured by nitrogen determinations on the solid material and the yields were calculated from relation 3*a*.
[b] n_{BO} in relation 3.
[c] Tested on plates with extract G as sole carbon and energy source.
[d] Gravimetric determinations.
[e] Unhydrolyzed cellulose powder.
[f] The lignin, after dialysis against water, was used in 0.1% concentration.
[g] No increase in the nitrogen concentration in the solid material was found during 100 h of incubation.

solid material. The stationary phase cannot be established by weight decrease measurements, since the cell mass decreased during this period (cf. Figs. 2 and 9). Thus such measurements gave no information regarding the yield in stationary phase.

Growth of A. fumigatus in Different Bark Fractions

To trace those substances in the bark that could function as carbon and energy sources for *A. fumigatus*, the different bark fractions (cf. Fig. 1) were tested for ability to support growth of the fungus. As shown in Table 4 the fatty acid containing residue D was a very poor substrate and at the last time of sampling (after 170 h of incubation) stationary phase had not yet been reached. Extract E, a complex mixture of hydrocarbons, phenols, and fatty acids, supported growth and the stationary phase was reached after 100 h. The yield was then about 50% of that on particulate bark. As extract E constitutes only 7.8% of unextracted bark (bark A) it is of minor importance for the growth on unextracted bark. High yields were obtained when the fungus grew on the water-soluble extract H, which probably contains carbohydrates of low molecular weight. In this case stationary phase was reached after 60–70 h. The yield determined by the amount of nitrogen in the solid material was 0.28 mg/mg substrate. Direct dry weight determinations gave 0.43 mg/mg substrate; difficulties in washing the fungus when growing in this extract probably resulted in an overestimation of the yield. Extract G as sole carbon and energy source did not promote any growth of *A. fumigatus* (Table 4). The results of Table 4 have been used to estimate which fractions of the bark contributed significantly to the growth of *A. fumigatus*. The result is summarized in Table 5.

Both extract G and residue D inhibited growth from spores on plates containing rich medium and different amounts of the respective fraction.

TABLE 5

Comparison of growth obtained on bark fractions[a]

Fraction[a]	Amount of bark fraction/ 100 mg bark	Growth, mg fungus/ 100 mg bark
A	100	15
D	2.2	0.0
E	7.8	0.6
G	14	0.0
H	11	3.1
I	65	10.4
D–I	100	14.1

[a] See fractionation procedure in Fig. 1.

When either fraction was present in 0.5% (w/v) concentration in rich medium, there was 90% inhibition, demonstrated by testing for colony formers (Grennberg and Nordström, unpublished).

Growth on Cellulose and Lignin

Since *A. fumigatus* is known to degrade cellulose (43, 51), it was tested for its ability to grow on cellulose powder. Only minor growth was observed on untreated cellulose powder when measured by nitrogen determination in the solid material during 170 h (Table 4). No growth was observed by spreading a spore suspension on agar plates with untreated cellulose as the sole carbon and energy source. Minor growth was seen when spores were inoculated on hydrolyzed cellulose (Walseth cellulose) plates. The increase in cell mass, however, was too small to be recorded by measuring nitrogen consumption in the medium during 100 h. No growth was observed when lignin was used as a carbon and energy source (Table 4). However, *A. fumigatus* was able to metabolize small monomeric model substances of lignin such as dehydroisoeugenol, phenol, and lignosulfonic acid but not vanillin or vanillic acid.

Residue I was subjected to lignin and carbohydrate analyses before and after 100 h of fungal growth to discover which substances in the residue had been utilized. The fungus (50 mg, washed and dried), grown for 70 h in starch minimal medium, was analyzed in the same way for carbohydrate content. Since no carbohydrates were found in the fungus the cell walls of the fungus were probably not hydrolyzed. This means that values obtained from mixtures of degraded bark and fungus only refer to the bark. As shown in Table 6 carbohydrates, but not lignin, are degraded by the fungus. *Aspergillus fumigatus* mainly consumed glucose (0.136 mg/mg bark) but other carbohydrates were also degraded; in all, 0.218 mg carbohydrate/mg residue I was consumed. As the weight difference of the solid material before and after degradation was 0.107 mg/mg residue I the weight contributed by the fungus was 0.111 mg/mg residue I (cf. Discussion).

TABLE 6

Carbohydrate and lignin concentrations of bark before and after degradation of *A. fumigatus*

Substance	% dry wt.		Amount used, mg
	Undegraded[a] bark	Degraded[a] bark	
Araban	8.2	4.8	32
Xylan	9.1[b]	5.0[b]	38[b]
Mannan	4.5	4.9	2
Galactan	2.9	1.8	10
Glucan	38.3	24.6	136
Klason lignin	35.5	44.2	−23
Acid soluble lignin	1.1	2.1	−6
Total	99.6	87.4	199

[a] Residue I (1000 mg) in M63 (100 ml) was incubated for 100 h without and with *A. fumigatus*; the solid material was collected on glass filters and washed, dried, and weighed. The dry weights were 804 mg and 697 mg respectively.

[b] These values may not be valid because of high background.

Discussion

Isolation and Properties of the Bark-degrading Fungus A. fumigatus

Many organisms were found to be able to use bark as sole carbon and energy source (Boman and Nordström, unpublished). One rapidly growing fungus, *A. fumigatus*, was chosen for further investigation. The species *A. fumigatus* has been reviewed (43) as well as its genetic system (10, 50). The genetics of *Aspergillus* has been investigated (42). *Aspergillus fumigatus* and *Gilmaniella humanicola* are predominant decomposers of bark (39). As *A. fumigatus* can be pathogenic to man (19) it should not be grown on a large scale. However, it has been used in this investigation as a model bark-degrading microorganism. Other bark-degrading microorganisms can probably be easily isolated by making minor changes in the growth conditions, e.g. pH, temperature, or medium. Other fungi have been shown to be able to degrade bark in pure culture (31). Spores of *A. fumigatus* were used as inoculum in growth experiments in defined media. The spores germinated and formed short hyphae which increased 50- to 100-fold in mass during the first 25 h of incubation (Table 2). Spores of *A. nidulans* germinate after a short lag and the cells grow exponentially for four or five doublings (46). Assuming that there was exponential growth in the experiments reported in Table 2, the generation time was 3–5 h, which is comparable to what has been found for *Aspergillus*, *Penicillium*, and *Mucor* species in similar media (20, 46, 54). After this first period the fungus formed pellets, a common behavior of fungi growing in submerged cultures (34). After the first 25 h of growth the increase in cell mass was linear in the defined media used. Linear growth was also obtained in bark media. Thus during the main period growth was linear; mycelial fungi grow linearly in submerged culture or show even more complicated kinetics (34). The linear increase in cell mass was the same for starch-grown cells as for bark-grown cells, i.e. bark A, residue AW and I (cf. Figs. 2 and 6).

Calculations of Growth in Bark Medium by Nitrogen Determinations

In particulate bark media it was not possible to measure the growth of *A. fumigatus* by direct dry weight determinations, since the fungus and the bark can not be separated. In other investigations on bark, weight decrease of the solid material (31, 38) or evolution of carbon dioxide (2, 4, 5, 7, 14) has been used as an indirect measure of microbial activity. As is apparent from Fig. 9, the curve for the weight loss of the solid material and that of the fungal growth do not coincide. Furthermore, in the stationary phase the dry weight continues to decrease because of weight loss of the fungus (cf. Fig. 2). Hence, measurements of weight decrease is not a direct measure of fungal activity or growth.

To evaluate the bark as a carbon and energy source for the fungus it was desirable to know the maximal yield expressed as fungus obtained per milligram bark. Various methods that might be correlated to the dry weight were tried, i.e. protein, nitrogen, and phosphorus determinations, measurement of differences in spectra before and after growth in the bark medium, and measurements of the weight decrease in the solid material. Nitrogen determinations proved to be useful in determination of fungal growth since the nitrogen concentration in *A. fumigatus* was mainly constant throughout the growth cycle (cf. Fig. 2) and was the same when the fungus was grown at different concentrations of the carbon source (cf. Table 3 and Fig. 4). Furthermore, the difference in nitrogen concentration between the fungus and the bark was large enough to permit estimations of fungal growth on bark (cf. Table 2).

Growth of *A. fumigatus* was followed by nitrogen analyses of solid material or by measurement of the nitrogen consumption in the particle-freed growth medium. The amount of cell material was calculated using relations 3 and 8 respectively. These relations are based on three

TABLE 7

Variation in yield[a] of *A. fumigatus* on residue I depending on various assumptions

		Fungal yields (mg/mg) based on nitrogen determinations in:	
Assumptions	Other possibilities	solid material[b]	medium[c]
1[d], 2[e], and 3[f] valid	None	0.16	0.20
1[d] not valid	(A) The nitrogen in the bark is completely dissolved or metabolized. $n_{BR} = 0$	0.21	0.24
	(B) The nitrogen in the bark is not dissolved or metabolized. $n_{BS} = 0$	0.14	0.16
2[e] not valid	(A) The nitrogen concentration is higher; e.g. $n_F = 7.0\%$ (solid) or 8.1% (medium)	0.14	0.18
	(B) The nitrogen concentration is lower; e.g. $n_F = 5.5\%$ (solid) or 6.4% (medium)	0.18	0.23
3[f] not valid	(A) The fungal yield is lower; e.g. $k = 0.4$	0.16	0.21

[a] The dry weight in stationary phase.

[b] *A. fumigatus* was grown in bark medium (residue I in M63). Total nitrogen determinations and dry weight determinations were performed on the solid material, and the yields were calculated from relation 3 (the same experiment as illustrated in Figs. 6 and 9).

[c] *A. fumigatus* was grown in bark medium (residue I in M64, containing 0.205 mg nitrogen/ml medium). The nitrogen consumption was determined and the yields were calculated from relation 8 (the same experiment as illustrated in Fig. 7).

[d] The bark is broken down homogeneously, *i.e.* $n_{BO} = n_{BR} = n_{BS}$.

[e] The nitrogen concentration in the fungus is the same when grown in bark medium as when grown in starch medium, i.e. $n_F = 6.3\%$ and 7.3% for determinations on the dried fungus and from the nitrogen consumption respectively.

[f] The fungal yield in milligrams per milligram available bark is the same as the fungal yield in starc minimal medium, i.e. $k = 0.5$.

assumptions (see Results section and Table 7). Unfortunately, it is not possible to directly verify any of these three assumptions. Therefore, the effect of variations in the assumptions on the calculated yield has been summarized in Table 7. If the assumption that bark is degraded homogeneously during fungal growth (assumption 1) is not valid, the calculated maximal variation in yield is 0.14 to 0.24 mg dry weight of fungus/mg of residue I. The alternatives of assumption 2 are less likely than the nitrogen concentration chosen for the calculations, namely that of starch. Both bark and starch are polymeric substrates and *A. fumigatus* was shown to utilize the polymeric carbohydrates of bark. Furthermore, the growth rate in bark medium was similar to that in starch medium and sporulation in starch medium and in bark medium occurred at about the same time and after the main growth period. Righelato *et al.* (45) have reported that a good carbon and energy source inhibits sporulation.

The value chosen for the economic coefficient (50%, assumption 3) is based on the finding that half of the carbohydrate degraded was converted to cell material (cf. Table 6). The value is fairly high (41). Furthermore the weight decrease of the solid material was 0.13 ± 0.01 mg when the fungal yield was 0.16 ± 0.01 mg/mg residue I (cf. Figs. 6 and 9).

In conclusion, the estimated fungal yield (0.16 mg dry weight/mg of residue I) is reasonable.

Thus growth of *A. fumigatus* in bark medium was measured either by measuring the amount of nitrogen and the dry weight of the solid material or by measuring the nitrogen consumed from the medium. The two methods give nearly the same result. The second method (nitrogen consumption) is simpler but is more uncertain, since an evaluation of evaporation is necessary. The general relations 3 and 8 are not restricted to bark media but are applicable to any medium in which the carbon and energy source is particulate. The method of quantification is especially useful when the growth parameter, i.e. nitrogen, is a constituent in the "particles" containing the carbon and energy source.

What Substances in the Bark Are Used as Substrates for A. fumigatus?

The cellular yield on unextracted bark was at least 15%. Major growth was obtained from the polymeric residue I (cf. Table 5). Significant contributions to the yield were also obtained from extract H, which probably contains low molecular weight carbohydrates. Growth on extracted and unextracted bark gave roughly the same yields of *A. fumigatus*, in agreement with observations on growth on pine bark by Kuhlman (3). However, spruce bark stored under water is reported to be a much poorer substrate than untreated bark (38). In both of the reported cases growth was estimated by weight decrease of solid material and submerged cultures were not used.

The polymeric residue I was analyzed before and after degradation by *A. fumigatus*. There was reduction in the amount of carbohydrate but not in the amount of lignin. Since glucose, arabinose, and xylose were consumed it can be concluded that cellulose and hemicellulose were used as substrates. This is in accordance with the fact that *A. fumigatus* is known to degrade cellulose (43, 51). However, the carbohydrates were not completely metabolized. This may be due to the presence of lignin, which may protect the carbohydrates from degradation (32). No reports have been found in the literature describing the analysis of bark for carbohydrates before and after degradation.

Growth Yields in Bark Media

The growth yields of *A. fumigatus* on bark were 16–20%. Applying the economic coefficient of 50%, this means that the fungus utilized 32–40% of the bark during 4 days of incubation. Other studies on bark degradation have shown that 10–20% of the bark was oxidized to carbon dioxide (3, 4, 5, 7, 14) or that there was a corresponding decrease in weight (31, 38). Moist bark was used as substrate in all investigations cited for pure fungal cultures (31) or mixed cultures (2, 4, 5, 7, 14, 38). Solid bark (31) or finely ground bark (2, 4, 5, 38) was used and the incubation time was of the order of months. In general 20% degradation or more was obtained on bark containing low amounts of extractive substances (2, 4, 5). In the works cited above it was not proven that the polymeric parts of the bark were degraded. Furthermore, the methods used for quantifying were indirect. The results reported in the present paper show that a pure fungal culture growing under submerged conditions can utilize bark efficiently and rapidly. *Aspergillus* *fumigatus* degraded 32–40% of the polymeric carbohydrates of the bark in 4 days of incubation with an economic coefficient of 50%.

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1. Alexander, M. 1961. Introduction to soil microbiology. John Wiley and Sons, Inc., New York and London.
2. Allison, F. E., and C. J. Klein. 1961. Comparative rates of decomposition in soil of wood and bark particles of several soft wood species. Soil Sci. Soc. Am. Proc. **25**: 193–196.
3. Allison, F. E., and R. G. Cover. 1960. Rates of decomposition of shortleaf pine sawdust in soil at various levels of nitrogen and lime. Soil Sci. **89**: 194–201.
4. Allison, F. E., and R. M. Murphy. 1962. Comparative rates of decomposition in soil of wood and bark particles of several hardwood species. Soil Sci. Soc. Am. Proc. **26**: 463–466.
5. Allison, F. E., and R. M. Murphy. 1963. Comparative rates of decomposition in soil of wood and bark particles of several species of pines. Soil Sci. Soc. Am. Proc. **27**: 309–312.
6. Ames, B. N., and D. T. Dubin. 1960. The role of polyamines in the neutralization of bacteriophage deoxyribonucleic acid. J. Biol. Chem. **235**: 769–775.
7. Aspitarte, T. R. 1959. Availability of nitrogen in ammoniated bark used as soil amendment. Diss. Abstr. **19**: 3084–3085.
8. Bailey, P., W. Liese, and R. Rörsch. 1968. Some aspects of cellulose degradation in lignified cell walls. *In* Biodeterioration of material. *Edited by* A. H. Walters and J. J. Elphick. Elsevier, London. pp. 546–557.
9. Berg, B., B. v. Hofsten, and G. Pettersson. 1972. Growth and cellulase formation by *Cellvibrio fulvus*. J. Appl. Bacteriol. **35**: 201–214.
10. Berg, C. M., and E. D. Garber. 1962. A genetic analysis of color mutants of *Aspergillus fumigatus*. Genetics, **47**: 1139–1146.
11. Bertani, G. 1951. Studies on lysogenesis. I. The mode of phage liberation by lysogenic *Escherichia coli*. J. Bacteriol. **62**: 293–300.
12. Bethge, P. O., R. Rådeström, and O. Theander. 1971. Kvantitativ kolhydratbestämning—en detaljstudie. Svenska Träforskningsinstitutets Meddelande Ser. B Nr 63 (AS B: 4 KA B: II) (Swedish Forest Products Research Laboratory Box 5604, S-114 86 Stockholm 5).
13. Bollen, W. B., and K. C. Lu. 1961. Microbial decomposition and nitrogen availability of reacted sawdust, bagasse, and coffee grounds. J. Agric. Food Chem. **9**: 9–15.
14. Bollen, W. B., and K. C. Lu. 1970. Sour sawdust and bark. U.S. For. Serv. Res. Pap. PNW, **108**: 2–13.
15. Boman, H. 1971. Vartåt barkar mikrobiologin i Umeå. Forsk. Framsteg, **5**: 24–25.
16. Bradstreet, R. B. 1965. The Kjeldahl method for organic nitrogen. Academic Press, New York and London.
17. Bye, A., W. M. Ashton, and H. K. King. 1968. A proposed mechanism for the conversion of *o*-coumaric acid to 4-hydroxy-coumarin in *Aspergillus fumigatus* Fresenius. Biochem. Biophys. Res. Commun. **32**: 94–97.
18. Cowling, E. B. 1970. Nitrogen in forest trees and its role in wood deterioration. Dissertation, Uppsala, Sweden.
19. Davies, B. D., R. Dulbecco, H. N. Eisen, H. S. Ginsberg, and W. B. Wood. 1968. *In* Microbiology. Hoeber Medical Division. Harper and Row, Publ., New York, Evanston, and London.
20. Dorn, G., and W. Rivera. 1966. Kinetics of fungal growth and phosphatase formation in *Aspergillus nidulans*. J. Bacteriol. **92**: 1618–1622.
21. Enebo, L. 1949. Symbiosis in thermophilic cellulose fermentation. Nature (Lond.), **163**: 805.
22. Enebo, L. 1954. Studies in cellulose decomposition by an aerobic thermophilic bacterium and two associated non-cellulolytic species. Dissertation, Stockholm, Sweden.
23. Eriksson, K-E., and U. Lindholm. 1971. Ligninets mikrobiella nedbrytning. Svsk. Papperstidn. **74**: 701–706.
24. Eriksson-Grennberg, K. G. 1968. Resistance of *Escherichia coli* to penicillins. II. An improved mapping of the *ampA* gene. Genet. Res. Engl. **12**: 147–156.
25. Gould, R. F. (*Editor*). 1969. Cellulases and their applications. Adv. Chem. Ser. **95**: 1–470.
26. Han, Y. W., and V. R. Srinivasan. 1968. Isolation and characterization of a cellulose utilizing bacterium. Appl. Microbiol. **16**: 1140–1145. 1–470.
27. Henningson, B. 1967. Microbial decomposition of unpeeled birch and aspen pulpwood during storage. Stud. For. Suecica Nr 54.
28. Jensen, W., K. E. Fremer, P. Sierila, and V. Wartiouaara. 1963. The chemistry of bark. *In* The chemistry of wood. *Edited by* B. L. Browning. John Wiley and Sons, Inc., New York. pp. 587–666.
29. Keilich. G., P. Bailey, and W. Liese. 1970. Enzymatic degradation of cellulose, cellulose derivatives and hemicelluloses in relation to fugal decay of wood. Wood Sci. Technol. **4**: 273–283.
30. Kirk, T. K. 1971. Effects of microorganisms on lignin. Annu. Rev. Phytopathol. **9**: 185–210.
31. Kuhlman, E. G. 1970. Decomposition of loblolly pine bark by soil-and root-inhabiting fungi. Can. J. Bot. **48**: 1787–1793.
32. Liese, W. 1970. Ultrastructural aspects of woody tissue disintegration. Annu. Rev. Phytopathol. **8**: 231–258.
33. Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. **193**: 265–275.
34. Mandels, G. R. 1965. Kinetics of fungal growth. *In* The fungi. Vol. I. *Edited by* G. C. Ainsworth and A. S. Sussman. Academic Press, New York and London. pp 599–612.

35. MINARI, O., and D. B. ZILVERSMIT. 1963. Use of KCN for stabilization of color in direct nesslerization of Kjeldahl digests. Anal. Biochem. **6**: 320–327.

36. NEAL, J. L., W. B. BOLLEN, and K. C. LU. 1965. Influence of particle size on decomposition of red alder and Douglas fir sawdust in soil. Nature (Lond.), **205**: 991–993.

37. NORIN, T., and B. WINELL. 1972. Extractives from the bark of common spruce, *Picea abies* L. Karst. Acta Chem. Scand. **26**: 2289–2296.

38. NYKVIST, N. 1969. Nedbrytningsförsök med bark av tall och gran. Statens Väginstitut, Stockholm, Specialrapport 75.

39. OTSUKA, H., and T. TAKE. 1970. A study on utilization of barks. Mem. Fac. Educ. Niigata Univ. **12**: 57–68.

40. PARDEE, A. B., F. JACOB, and J. MONOD. 1959. The genetic control and cytoplasmic expression of "inducibility" in the synthesis of β-galactosidase by *E. coli*. J. Mol. Biol. **1**: 165–178.

41. PERLMAN, D. 1965. The chemical environment for fungal growth. *In* The fungi. Vol. I. *Edited by* G. C. Ainsworth and A. S. Sussman. Academic Press, New York and London, pp. 479–489.

42. PONTECORVO, G., J. A. ROPER, L. M. HEMMONS, K. D. MACDONALD, and A. W. J. BUFTON. 1953. The genetics of *Aspergillus nidulans*. Adv. Genet. **5**: 142–238.

43. RAPER, K. B., and D. I. FENNELL. *(Editors.)* 1965. *Aspergillus fumigatus* group. *In* The genus *Aspergillus*. The Williams and Wilkins Co., Baltimore. pp. 238–268.

44. RAUTELA, G. S., and E. B. COWLING. 1966. Simple cultural test for relative cellulolytic activity of fungi. Appl. Microbiol. **14**: 892–898.

45. RIGHELATO, R. C., A. P. J. TRINCI, S. J. PIRT, and A. PEAT. 1968. The influence of maintenance energy and growth rate on the metabolic activity, morphology and conidiation of *Penicillium chrysogenum*. J. Gen. Microbiol. **50**: 399–412.

46. ROSENBERG, R. F., and M. KESSEL. 1967. Synchrony of nuclear replication in individual hyphae of *Aspergillus nidulans*. J. Bacteriol. **94**: 1464–1469.

47. RYAN, F. J., G. W. BEADLE, and E. L. TATUM. 1943. The tube method of measuring the growth rate of *Neurospora*. Am. J. Bot. **30**: 784–799.

48. SCHUBERT, W. J. 1964. Fungal degradation of softwood lignins. Chim. Biochim. Lignine Cellul. Hemicellul. Actes Symp. Intern. Grenoble, France. pp. 457–468.

49. SRIVASTAVA, L. M. 1964. Anatomy, chemistry and physiology of bark. Int. Rev. For. Res. **1**: 203–277.

50. STROMNAES, Ø., and E. D. GARBER. 1963. Heterocaryosis and the parasexual cycle in *Aspergillus fumigatus*. Genetics, **48**: 653–662.

51. STUTZENBERGER, F. J., A. J. KAUFMAN, and R. D. LOSSIN. 1970. Cellulolytic activity in municipal solid waste composting. Can. J. Microbiol. **16**: 553–560.

52. SUNDMAN, V., and L. NÄSE. 1971. A simple plate test for direct visualization of biological lignin degradation. Pap. Timber Helsinki, **53**: 67–71.

53. SUNDMAN, V., and L. NÄSE. 1972. The synergistic ability of some wood-degrading fungi to transform lignins and lignosulfonates on various media. Arch. Mikrobiol. **86**: 339–348.

54. TRINCI, A. P. J. 1969. A kinetic study of the growth of *Aspergillus nidulans* and other fungi. J. Gen. Microbiol. **57**: 11–24.

55. VOGEL, H. J., and D. M. BONNER. 1956. Acetylornithinase of *Escherichia coli*: partial purification and some properties. J. Biol. Chem. **218**: 97–106.

56. YAMAMOTO, Y., N, KIRIYAMA, and S. ARAHATA. 1968. Studies on the metabolic products of *Aspergillus fumigatus* (J-4). Chem. Pharm. Bull. (Tokyo), **16**: 304–310.